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Structure and Function of Vacuolar Na^+-Translocating ATPase in *Enterococcus hirae*

Yoshimi Kakinuma,[1,3] Ichiro Yamato,[2] and Takeshi Murata[2]

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A Na^+-translocating ATPase was discovered in a gram-positive bacterium *Enterococcus hirae*. Our biochemical and molecular biological studies revealed that this Na^+-ATPase belongs to the vacuolar-type enzyme. Purified Na^+-ATPase consisted of nine subunits: NtpA, B, C, D, E, F, G, I, and K; reconstituted proteoliposomes showed ATP-driven electrogenic Na^+ translocation. All these subunits were encoded by the *ntp* operon: *ntpFIKECGABDHJ*. The deduced amino acid sequences of the major subunits, A, B, and K (16 kDa proteolipid), were highly similar to those of A, B, and proteolipid subunits of vacuolar ATPases, although the similarities of other subunits were moderate. The *ntpJ* gene encoded a K^+ transporter independent of the Na^+-ATPase. Expression of this operon, encoding two transport systems for Na^+ and K^+ ions, was regulated at transcriptional level by intracellular Na^+ as the signal. Two related cation pumps, vacuolar Na^+-ATPase and F_0F_1, H^+-ATPase, coexist in this bacterium.

KEY WORDS: Na^+-ATPase; vacuolar ATPase; *Enterococcus hirae*.

INTRODUCTION

Proton circulation is widely utilized for energy transduction across the membrane, such as mitochondrial inner membrane, chloroplast thylakoid membrane, and bacterial cell membrane. The electrochemical concentration gradient of protons is generated by various primary proton pumps, such as H^+-linked electron transport systems and bacteriorhodopsin (Mitchell, 1976; Harold, 1977, 1986). The established gradient (proton potential) is utilized by a variety of H^+-consuming systems: ATP synthase, H^+-linked secondary active transport systems, and H^+-linked flagellar motor. On the other hand, several organisms living in high salinity or high pH have evolved a variety of primary sodium pumps. The electrochemical gradient of sodium is generated by Na^+-linked electron transport systems and decarboxylases in some bacteria (Dimroth, 1987). The generated Na^+ gradient (sodium potential) is utilized by Na^+consuming systems, such as various Na^+-linked secondary active transporters. In marine bacteria, as well as alkalophilic bacilli, the sodium potential is utilized by Na^+-linked flagellar motor (Imae and Atsumi, 1989). In *Propionigenium modestum*, the sodium potential generated by decarboxylase is converted to ATP by Na^+-linked ATP synthase (Laubinger and Dimroth, 1988). Sodium circulation is equally important for energy transduction and we now understand distribution of various Na^+-linked energy-transducing machineries. As described in this mini-review, vacuolar ATPase is found as a proton pump, which is distributed in various organelles and some plasma membranes of eukaryotes and bacteria, especially archaebacteria. In the fermentative eubacterium *Enterococcus hirae*, we found a vacuolar ATPase, which pumps sodium ions. This Na^+-ATPase plays a key role for sodium homeostasis of this bacterium. In this article, we summarize the molecular features and function of *E. hirae* vacuolar Na^+-ATPase.

[1] Faculty of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522.
[2] Department of Biological Science and Technology, Science University of Tokyo, 2641 Yamazaki, Noda-shi, Chiba 278-8510, Japan.
[3] Author to whom correspondence should be addressed.

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DISCOVERY

Sodium extrusion in bacteria is generally performed by secondary sodium/proton antiport system energized by the proton electrochemical potential across cell membrane (Harold, 1977, 1986). *Enterococcus hirae* lacking the respiratory chain was found to have an additional sodium extrusion system, Na^+-ATPase. Sodium extrusion against a concentration gradient was initiated by addition of glucose (Fig. 1). *N*, *N′*-dicyclohexylcarbodiimide (DCCD) inhibits the F_0F_1, H^+-ATPase (F-ATPase) activity, which generates the proton potential. This sodium extrusion was observed even when the proton potential had been totally dissipated by the presence of DCCD, a protonophore, and valinomycin (Fig. 1A), suggesting that it was not exerted by the secondary sodium/proton antiporter. Since this proton potential-independent sodium extrusion was also observed in arginine-adapted cells, in which arginine-deiminase pathway was induced, ATP, the common high-energy compound in metabolism of glucose and arginine, is likely to be the energy for this reaction (Heefner and Harold, 1980). ATP-driven Na^+ uptake and Na^+-stimulated ATP hydrolysis were also observed by the everted membrane vesicles in the presence of DCCD and the ionophores (Heefner and Harold, 1982). In mutant Nak1 (Kakinuma and Igarashi, 1990a), which is now considered to be defective in the major subunit of the Na^+-ATPase, the proton potential-independent sodium extrusion was not observed (Fig. 1B); the Na^+-ATPase activity was not observed by the vesicles of Nak1. All these results suggest that a Na^+-translocating ATPase exists in the cell membrane of *E. hirae*. It is now accepted that *E. hirae* has two sodium extrusion systems: the Na^+-ATPase and the proton potential-driven Na^+/H^+ antiporter (Kakinuma and Igarashi, 1989).

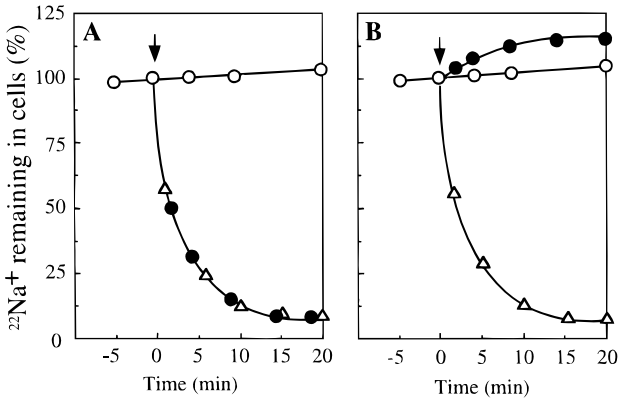


Fig. 1. Na^+ extrusion from (A) *E. hirae* ATCC 9790 and (B) Nak1 (Na^+-ATPase mutant). Cells were suspended in 50 m*M* K^+-HEPES (*N*-2-hydroxyethylpiperazine-*N′*-2-ethanesulfonic acid) buffer (pH 7.0) containing 400 m*M* K^+-maleate; 20 m*M* $^{22}NaCl$ was added. After 60 min, the suspension was divided into aliquots and 10 m*M* glucose was added at 0 min. ○, No glucose; △, glucose; ●, 0.4 m*M* DCCD, 10 μ*M* tetrachlorosalicylanilide and 10 μ*M* valinomycin at -5 min and glucose at 0 min.

STRUCTURE

One decade after the discovery of the Na^+-ATPase, the molecular information on this ATPase was obtained (Kakinuma and Igarashi, 1990c, d) and that *E. hirae* Na^+ ATPase is a complex consisting of membrane-peripheral and membrane-embedded protein(s). In an effort to purify the Na^+-ATPase from everted membrane vesicles, we found that the Na^+-stimulated ATP hydrolytic activity of the vesicles was easily lost by treatment with ethylenediaminetetraacetic acid (EDTA). However, the Na^+-ATPase activity was fully recovered by addition of an excess amount of Mg^{2+} in this EDTA-treated membrane suspension; the component essential for the activity of this enzyme was released (Kakinuma and Igarashi, 1990c). This essential component in the EDTA extract was identified by polyacrylamide gel electrophoresis (PAGE)(Fig. 2). ATP hydrolytic activity was found in the gel by active staining, just underneath the F_1 moiety of F-ATPase also released. This protein was not detected in the EDTA extract of mutant Nak1 and was recovered in Nak1R, a revertant of Nak1 (Fig. 2, lanes 2 and 3)(Kakinuma and Igarashi, 1990c). It is now clear that this mutant is unable to produce one of the major subunits (65 kDa A subunit) of this enzyme complex; the catalytic V_1 moiety was not assembled. Later, this ATPase was purified (Kakinuma and Igarashi, 1994); the molecular mass of this ATPase was about 400 kDa consisting of 65-, 56-, and 29-kDa polypeptides with a probable stoichiometry of 3:3:1. Rabbit antiserum against the purified ATPase inhibited the Na^+-stimulated ATP hydrolytic activity of the membranes (Kakinuma and Igarashi, 1994). This ATPase was hereafter assigned as the Na^+-ATPase catalytic component.

Enterococcus hirae Na^+-ATPase had been circumstantially considered to be the vacuolar-type (Kakinuma and Igarashi, 1990c; Kakinuma *et al.*, 1991) and direct evidence of the *E. hirae* Na^+-ATPase as V-ATPase was obtained by cloning of the *ntp* operon: *ntpFIKECGABD(H)J* encoding this enzyme (Kakinuma *et al.*, 1993; Takase *et al.*, 1993, 1994)(Fig. 3).

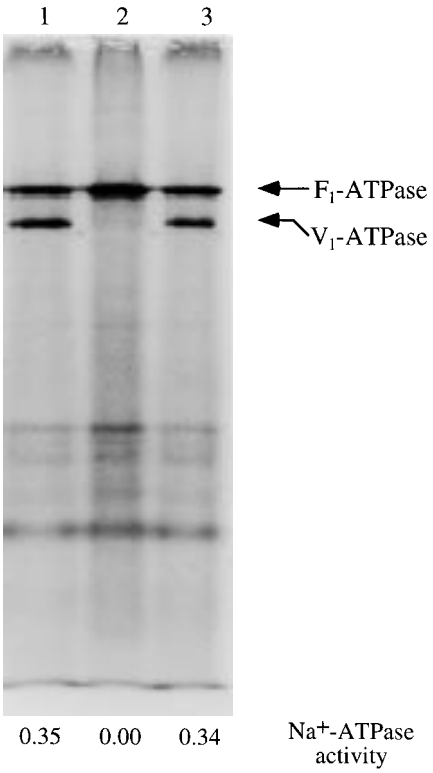
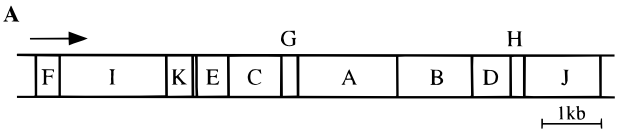


Fig. 2. PAGE of EDTA extracts of *E. hirae* membranes, prepared from membrane vesicles of *E. hirae* strains. The Na^+-ATPase activities of each of the vesicles are shown underneath. Lanes 1, 9790 (Parent); 2, Nak1 (Na^+-ATPase mutant); 3, Nak1R (revertant of Nak1). The arrows indicate F_1- and V_1-ATPase.



B

E. hirae Na^+-ATPase subunit	(kDa)	*S. cerevisiae* protein	(kDa)	Identity	[similarity] (%)
F	(14)	Vma10p	(13)	29	[46]
I	(76)	Vph1p	(100)	16	[37]
K	(16)	Vma3p	(17)	25	[51]
E	(23)	Vma4p	(27)	22	[39]
C	(38)	Vma6p	(36)	15	[36]
G	(11)	Vma7p	(14)	26	[52]
A	(66)	Vma1p	(69)	50	
B	(51)	Vma2p	(60)	53	
D	(27)	Vma8p	(32)	23	[48]
H	(7)	-		-	
J	(49)	Trk2p	(101)	27	[54]

Fig. 3. Organization of *E. hirae* Na^+-ATPase (*ntp*) operon. (A). Gene organization. The arrow indicates transcriptional direction. (B) Similarities between Na^+-ATPase subunits (ntp gene products) and *S. cerevisiae* proteins.

The 65-, 56-, and 29-kDa subunits of the purified ATPase were assigned to NtpA, NtpB, and NtpD gene products, respectively. Furthermore, extensive gene-disruption experiments (Solioz and Davies, 1994; and our group's unpublished data) indicate that all these *ntp* genes, except for the *ntpH* and *ntpJ* genes, are required for expression of the Na^+-ATPase; the *ntpJ* gene encodes a component of the K^+ transport system (KtrII system) (Murata *et al.*, 1996a), and it is doubtful the mini *ntpH* gene (183 bp) is the open reading frame. The deduced amino acid sequences of these Ntp gene products revealed a striking similarity between Ntp subunits and the eukaryotic V-ATPase subunits, especially of yeast (Fig. 3B). Three major subunits 65-kDa NtpA and 56-kDa NtpB subunits of the catalytic V_1 portion and the 16-kDa NtpK (proteolipid) subunit, are highly homologous to those of V-ATPases. The amino acid cluster, such as the motif GlyXXXXGly-LysThr/Ser, which are specifically conserved in the sequences of nucleotide binding proteins (Walker *et al.*, 1982), is found in the sequence GlyProPheGlyAlaGlyLysThr of NtpA subunit; a similar sequence, GlyThrGlyAlaGlyLysThr, is also found in the NtpB subunit (Takase *et al.*, 1993). The NtpK proteolipid subunit, which constitutes the putative ion pathway in the V_0 moiety, has DCCD-reactive glutamic acid residue (Glu139) in its fourth membrane-spanning domain, likely derived from tandem duplication of the c subunit of F-ATPase (Kakinuma *et al.*, 1993). Other Na^+-ATPase subunits are moderately similar to the corresponding ones of yeast V-ATPase. The similarity of NtpC, NtpE, and NtpI to the corresponding yeast V-ATPase subunits Vma6p, Vma4p, and Vph1p, respectively, were less prominent, but some amino acid clusters conserved among corresponding subunits in eukaryotic vacuolar ATPases are identified in the sequences.

The Na^+-ATPase was purified from the membranes of cells in which the amount of Na^+-ATPase was amplified by introducing plasmid harboring this *ntp* operon (Murata *et al.*, 1997)(Fig. 4A). Purified Na^+-ATPase consists of nine polypeptides, all of which were assigned to the Ntp gene products, except for *ntpH* and *ntpJ*. Densitometric analysis of SDS-PAGE staining of the purified enzyme suggested that the A,B,I,C,D,E,K,F,G subunits occurred in molar ratio of 3:3:1–2:1:1:3:1:4–6:1:1. Six hydrophilic subunits, A, B, C, D, E, and F subunits were released from the membrane-embedded V_0 portion by EDTA treatment (unpublished data), probably constituting the V_1 moiety; release of the smallest hydrophilic subunit G is

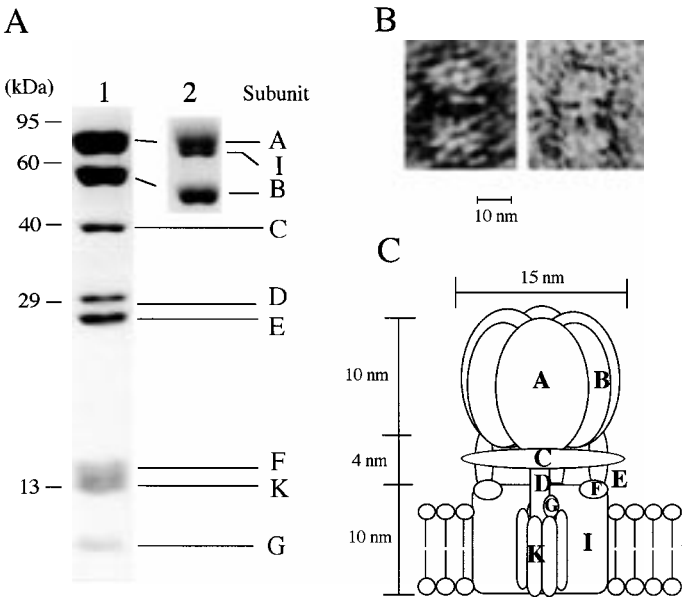


Fig. 4. Structure of *E. hirae* Na^+-ATPase. (A) SDS-PAGE of purified ATPase. Electrophoresis was performed by 12.5% gel (lane 1) and 10% gel (lane 2) and stained with Coomassie brilliant blue R-250. (B) Molecular image by negative staining. (C) Schematic model of the ATPase complex.

not identified. The V_0 moiety likely consists of two hydrophobic subunits: I and K. The projected structure of purified V_0V_1 Na^+-ATPase complex was analyzed by electron microscopy (Fig. 4B). A central flat stalk is observed in the interface that connects the headpiece V_1 and membrane-bound part V_0 of the complex. The speculative molecular model of *E. hirae* Na^+-ATPase is illustrated (Fig. 4C). Reconstituted proteoliposomes of purified ATPase showed ATP-driven Na^+ transport, which was accelerated by valinomycin and a protonophore, but blocked by monensin, suggesting that it is electrogenic (Fig. 5A)(Murata *et al.*, 1997). This bacterium has not only F-ATPase but V-ATPase, functionally coexisting in the same cell membrane of this bacterium (Fig. 2).

CATALYTIC PROPERTIES

The Na^+-stimulated ATPase activity of the membrane vesicles was maximal at pH 8.5–9.0, but not detectable at pH 6.0; the pH profile corresponds well to its role in this bacterium's sodium homeostasis at high pH (Kakinuma and Igarashi, 1989). Membrane ATPase was also stimulated by Li^+ but not by K^+ and Ca^{2+}. The ATP hydrolytic activity of purified enzyme absolutely requires Na^+ or Li^+ at pH 9.0 (Murata *et al.*, 1997); the kinetics of ATP hydrolysis showed at least two different affinities for Na^+ (K_m values of 20 μM and 4 mM) or Li^+ (K_m values of 60 μM and 3.5 mM), and probably one more (Fig. 5B). These different affinities for cations is probably related with the mechanism, but its meaning remains unsolved. It is interesting that the hydrolytic activity of purified enzyme did not depend on Na^+ or Li^+ at low pH (Fig. 5C). The activity at pH 6 without Na^+ corresponds to the activity at pH 9 with Na^+. It is very likely that the *E. hirae* Na^+-ATPase transports proton instead of Na^+ at low pH and this possibility is now under investigation. The affinity of this enzyme for ATP is 0.5 mM in purified and in membrane-bound forms (Murata *et al.*, 1997).

Enterococcus hirae Na^+-ATPase was discovered as the DCCD-insensitive one (Heefner and Harold, 1980, 1982). However, it is now clear that the activity of Na^+-ATPase is inhibited by this reagent (Murata *et al.*, 1997). A glutamic acid residue (Glu-139) in the NtpK proteolipid of the complex is likely covalently modified with DCCD. We found that the reaction of DCCD with the Na^+-ATPase is blocked by the presence of Na^+ or Li^+. Na^+ or Li^+ competes with DCCD, probably at the binding site: Glu-139. In all previous experiments where DCCD-insensitive Na^+ extrusion activity was observed, this reagent was added after Na^+ loading to block the generation of the proton gradient by the

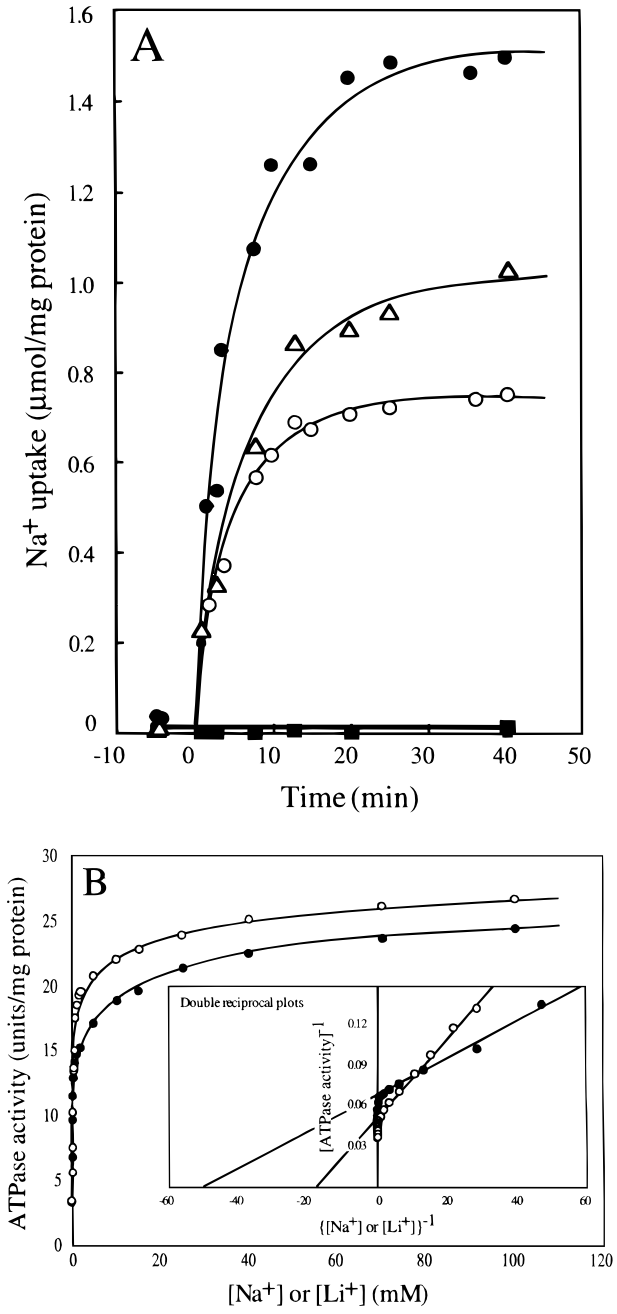


Fig. 5. Catalytic features of *E. hirae* Na^+-ATPase. (A) Na^+ uptake into proteoliposomes reconstituted with purified Na^+-ATPase. $^{22}Na^+$ uptake was started by the addition of 5 m*M* ATP at 0 min. Ionophores (25 μ*M*) were added at 10 min before the addition of ATP. ○, Control; ●, valinomycin; △, CCCP; ■, monensin. (B) Effect of salt concentration. The assay was performed at pH 9 at various concentrations of NaCl (○) or LiCl (●). Under the experimental conditions, 5–10 μ*M* Na^+ was present as contaminant. The inset shows the double reciprocal plots of ATP hydrolytic activities versus salts. (C) pH profile. The ATP hydrolytic activities of purified enzyme were measured in the absence (○) or the presence (●) of 25 m*M* NaCl.

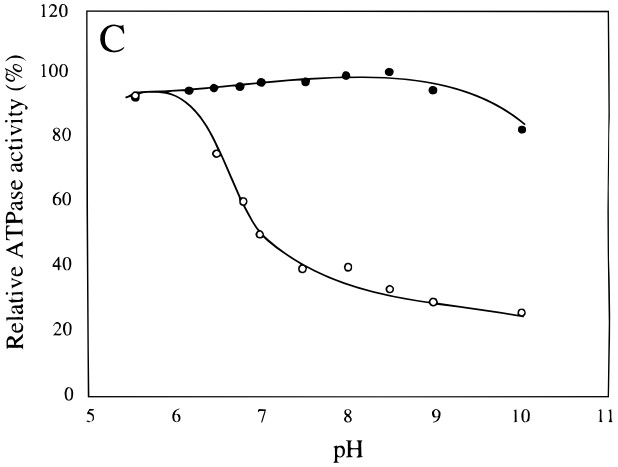


Fig. 5. Continued

H^+-ATPase. No proton gradient-independent Na^+ extrusion was observed when DCCD was added before Na^+ loading (unpublished data).

Destruxin B, the peptide antibiotic, which possibly attacks the V_1 catalytic portion of V-ATPase (Muroi *et al.*, 1994), was effective to *E. hirae* Na^+-ATPase as well as eukaryotic V-ATPases. However, the effect of the macrolide antibiotic bafilomycin A_1 (Bowman *et al.*, 1988), which probably attacks the V_0 portion of eukaryotic V-ATPase (Zhang *et al.*, 1994), was insignificant to bacterial V-ATPase; concanamycin A, another macrolide antibiotic, did not inhibit *E. hirae* Na^+-ATPase at 5 m*M*. Recently, it was shown that two proteolipid subunits, Vma11p and Vma16p, are also essential for the activity and assembly of yeast V-ATPase (Hirata *et al.*, 1997); more than 13 subunits build the V-ATPase, in *S. cerevisiae* and, as well, in *Caenorhabditis elegans* (Oka *et al.*, 1997). Indeed, the structure of bacterial V-ATPase and eukaryotic V-ATPase are fundamentally homologous, but the fine structures are distinct, probably reflecting in the different sensitivity to the antibiotics. The effect of nitrate on eukaryotic V-ATPase is explained by its chaotropic effect; the V_1 catalytic moiety is dissociated from the V_0 membrane sector. *Enterococcus hirae* Na^+-ATPase as well as archaebacterial V-ATPase was inhibited by nitrate, but inhibition occurred at a much lower concentration, and not as a chaotropic effect (Kakinuma and Igarashi, 1990c). *Enterococcus hirae* Na^+-ATPase was inhibited by *N*-ethylmaleimide (NEM), but the K_i value (0.15 m*M*) is higher than that for eukaryotic V-ATPase at micromolar order. It is proposed that the cysteine residue in the Walker A motif (GlyXXXCysGly-LysThr) conserved in the A subunit of eukaryotic

V-ATPase is attacked by the sulfhydryl reagent (Feng and Forgac, 1992). The corresponding amino acid residue is alanine (Ala236) in the NtpA subunit of *E. hirae* Na^+-ATPase. Replacement of the Ala236 to cysteine brought about much higher sensitivity to NEM (Takase, 1997).

MECHANISM

There must be a common energy-transducing principle between V- and F-ATPase molecules. An energy transfer occurs between ATP hydrolysis at three catalytic sites on the V_1 moiety and multiple ion translocations probably through the proteolipid of the V_0 portion in the complex. A rotation catalysis mechanism (Boyer, 1997), which is experimentally verified in F-ATPase, is applicable to V-ATPase. Since the D subunit makes the "core" complex with A and B subunits in purified V_1-ATPase (Kakinuma and Igarashi, 1994), D might mediate an energy transfer in rotation catalysis, like the γ subunit of F-ATPase (Boyer, 1997), although the similarity between the V-ATPase D subunit and the γ subunit of F-ATPase was insignificant. In rotation catalysis, the next important question is about the mechanism in the membrane sector, i.e., how rotation within the membraneous subunits is coupled with ion flow. The ATP hydrolysis in the isolated V_1 moiety was not stimulated by Na^+ (Kakinuma and Igarashi, 1994); direct interaction between the V_1 and V_0 moieties is the prerequisite for Na^+-stimulated ATPase activity (Kakinuma and Igarashi, 1990d). Furthermore, the DCCD reaction on the 16-kDa proteolipid is blocked by Na^+ or Li^+ (Murata *et al.*, 1997). The ion binding site is thus implied to be in the V_0 moiety, likely at Glu-139 of NtpK proteolipid, corresponding to an acid residue in the c proteolipid of F-ATPase. Ion movement in the F_0 sector of F-ATPase has been extensively investigated (Fillingame, 1997); P. Dimroth's group takes advantage of Na^+-coupled F-ATPase of *P. modestum* for the analysis of the mechanism (Dimroth *et al.*, 1998; Kaim and Dimroth, 1998).

PHYSIOLOGY OF THE Na^+-ATPase OPERON

NtpJ as K^+ Transporter

Before finding out the structural information of the Na^+-ATPase by gene cloning, Kakinuma and Harold presented a hypothesis that this ATPase may be $Na^+(K^+)$-ATPase (Kakinuma and Harold, 1985). Potassium uptake via the KtrII K^+ transport system, independent of the proton potential, was coincident to the activity of Na^+-ATPase, which functions to expel Na^+ by exchange for K^+. This mechanism was briefly described elsewhere (Kakinuma and Igarashi, 1989). In this context, the *ntpJ* gene, cotranscribed with other Na^+-ATPase subunit genes as a tailed cistron of the *ntp* operon, is interesting (Fig. 3A). The *ntpJ* gene encodes a 49-kDa hydrophobic protein having at least ten membrane-spanning domains, resembling Trk1p or Trk2p K^+ transporter in yeast (Takase *et al.*, 1994). Since the KtrII activity was missing in the *ntpJ*-disrupted *E. hirae* strain, it is clear that this gene encodes the component of KtrII K^+ uptake system (Murata *et al.*, 1996a). However, the NtpJ protein was separable from the Na^+-ATPase complex by centrifugation (Murata *et al.*, 1997) and the activity of Na^+-ATPase was, as usual, in the disrupted strain. The KtrII K^+ uptake system is thus not mechanically linked with the Na^+-ATPase complex and the K^+/Na^+ exchange model of Na^+-ATPase was withdrawn. Although the mechanism of KtrII (NtpJ) transport system is unsettled, it is likely a secondary Na^+/K^+ symporter; the *ntp* operon is an interesting one, which encodes two important transport systems for Na^+ and K^+ homeostasis.

Gene Expression

Enterococcus hirae Na^+-ATPase level in a cell was not constant (Kinoshita *et al.*, 1984). The sodium ATPase was induced when cells were grown on media rich in sodium, particularly under conditions that limit the generation of a proton gradient (Kakinuma and Igarashi, 1989, 1990b). Proton potential-driven sodium extrusion via the Na^+/H^+ antiporter is negligible in all these culture conditions, suggesting that an increase in the cytoplasmic sodium level serves as the signal for the induction. Western and northern blotting experiments revealed substantial correlation of the amount of Na^+-ATPase and expression of the *ntp* operon (Murata *et al.*, 1996b). Even under limited Na^+ concentrations, monensin or gramicidin D, rendering the membrane permeable to Na^+, significantly increased the amount of Na^+-ATPase and the mRNA for the *ntp* operon. All these data are explained by a hypothesis that the Na^+-ATPase is induced at transcriptional level by an increase in the cytoplasmic Na^+ concentration as the signal, presumably via the cytoplasmic Na^+-sensing system. The wild-type strain in the presence

of a protonophore or H^+-ATPase mutant AS25 did not grow in K^+-limited medium; K^+ accumulation did not occur. The addition of sodium ions to the medium recovered the internal K^+ level and the cell growth; sodium dependence in K^+ accumulation and cell growth were not observed in mutants defective in Na^+-ATPase subunit or NtpJ (Kawano *et al.*, 1998). These results suggest that expression of the *ntp* operon coding for the KtrII system and vacuolar Na^+-ATPase is indispensable for Na^+-dependent K^+ accumulation and cell growth at low proton potential.

DISTRIBUTION OF Na^+-ATPase

A Na^+-translocating ATPase, recently discovered in a thermophilic *Clostridium fervidus*, is very likely to be the vacuolar type, judging from the enzymatic and biochemical properties of the purified enzyme (Honer zu Bentrup *et al.*, 1997). Furthermore, the enzymatic features of Na^+-stimulated ATPase observed in *Mycoplasma mycoides*, a parasitic glycolytic organism, is virtually similar (Benyoucef *et al.*, 1982) and a Na^+-stimulated ATPase of *Acholeplasma laidlawii* seems to be of the V type judging from its subunit composition (Lewis and McElhaney, 1983). The occurrence of vacuolar Na^+-ATPase in other streptococci has not been well investigated. We have found the Na^+-stimulated ATPase activity and the immuno-reacted materials with anti-*E. hirae* V_1-ATPase serum in the everted membrane vesicles of some *Lactococcus lactis* strains (unpublished data). Some related streptococci, such as *L. lactis* and *Streptococcus bovis*, appear to have evolved Na^+-ATPase, although it may be less prominent. An electrogenic Na^+-ATPase has been reported in *P. modestum*, *Acetobacterium woodii* (Reidlinger and Muller, 1994), and *Methanosarcina mazei* (Becher, and Muller, 1994), etc., but these enzymes belong to the F-ATPase class.

CONCLUSION

Enterococcus hirae is the organism that has been clearly proved that functional F- and V-ATPases coexist on the same plasma membrane. However, coexistence of V- and F-ATPases in one bacterial cell is now not exceptional; distribution of both ATPases is also reported in *M. mazei* (Becher and Muller, 1994). In this bacterium, F-ATPase transports Na^+ and V-ATPase transports H^+. It is believed that V-ATPase diverged from F-ATPase relatively early in evolution. It is interesting how the ATPase genes are acquired by bacteria as a result of horizontal transmission and how the cation specificity, i.e., Na^+ or H^+, is altered in the course of adaptation to the environment. In addition to the molecular evolutional significance of vacuolar Na^+-ATPase in this bacterium, the sodium-coupled enzyme is expected to be very useful in the investigation of the mechanism of the energy-coupling ion of these energy-transducing systems. In addition, bacterial vacuolar ATPase should show the essential architecture as V-ATPase. *Enterococcus hirae* Na^+-ATPase is a useful system in the understanding of the fundamental structure and mechanism of ion translocation by V-ATPases.

ACKNOWLEDGMENTS

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